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SHORTER NOTES

6-*C*-β-Cellobiosylisoscutellarein-8-methyl ether, a new flavonoid from *Pteris vittata*.—In spite of the fact that analyses of fern flavonoids are of chemotaxonomic interest, data relating to flavonoids of some fern families (e.g. Pteridaceae) are limited. Previous work on the flavonoids of *Pteris vittata* L. has led to the identification of luteolinidin 5-*O*-glucoside by Harborne (Phytochemistry 5: 589–600, 1966) and acid hydrolysis of extracts of this fern led to the identification of kaempferol, quercetin, leucocyanidin and leucodelphinidin by Voirin (Ph. D. thesis, University of Lyon, p. 151, 1970); very recently 3-*C*-(6‴-*O*-acetyl-β-cellobiosyl) apigenin has been isolated from *Pteris vittata* L. by Imperato and Telesca (Amer. Fern J., 89:217–220,1999).

The present paper deals with the isolation of three flavonoid glycosides (I–III) from *Pteris vittata* growing in the Botanic Garden of the University of Naples (Italy). This fern was collected and identified by Dr. R. Nazzaro (University of Naples); a voucher specimen (149.001.001.01) has been deposited in the Herbariun Neapolitanum (NAP) of the University of Naples.

Flavonoids I-III were isolated by preparative paper chromatography in BAW (*n*-butanol-acetic acid-water, 4:1:5, upper phase), 15% HOAc (acetic acid) and BEW (*n*-butanol-ethanol-water, 4:1:2.2) from an ethanolic extract of aerial parts of *Pteris vittata* L.. Further purification was carried out by Sephadex LH-20 column chromatography, eluting with methanol.

R_f values on Whatman No 1 paper (0.33 in BAW; 0.56 in 15% HOAc), color reactions (brown to yellow in UV + NH_3) and ultraviolet spectral analysis in the presence of the customary shift reagents (λ_{max} (nm) (MeOH) 273, 301 (sh), 332; +NaOMe 283, 334, 400 (increase in intensity); +NaOAc 282, 306 (sh), 383; +$AlCl_3$ 279, 304, 348, 385, +$AlCl_3$/HCl 280, 304, 344, 383 were consistent with flavonoid (I) being a flavone glycoside with free hydroxyl groups at position 5 (shifts with $AlCl_3$ and $AlCl_3$/HCl), 7 (shift with NaOAc) and 4′ (shift with NaOMe). Acid hydrolysis (2N HCl/MeOH (1:1); 1 hr at 100°C) gave D-glucose and two flavonoids (IV and V) which showed free hydroxyl groups (detected by UV spectral analysis) at position 5, 7 and 4′ and behaved as flavonoid glycosides in paper chromatography; $FeCl_3$ oxidation of both flavonoids IV and V gave D-glucose. These results suggest that the isolated flavonoid (I) is a *C*-glycosylflavone in which the hydrolyzable D-glucose is not linked to phenolic hydroxyl groups whereas flavonoids IV and V may be a pair of Wessely-Moser isomers. Kuhn methylation (methyl iodide in dimethylformamide in the presence of silver oxide; 18 hr in the dark with stirring) of flavonoid (I) gave a permethyl (PM) ether wihich showed an EI-mass spectrum (MS) with $[M]^+$ at m/z (% base peak) 764 (4) and fragment ions at 749 (8), 733 (10), 718 (12), 705 (6), 691 (7), 675 (8), 663 (12), 648 (15), 633 (8), 623 (14), 603 (21), 529 (30), 515 (21), 410 (18), 399 (37), 397 (51), 383 (100), 368 (33), 341 (36), 314 (33), 287 (63). The molecular ion of PM flavonoid (I) (m/z

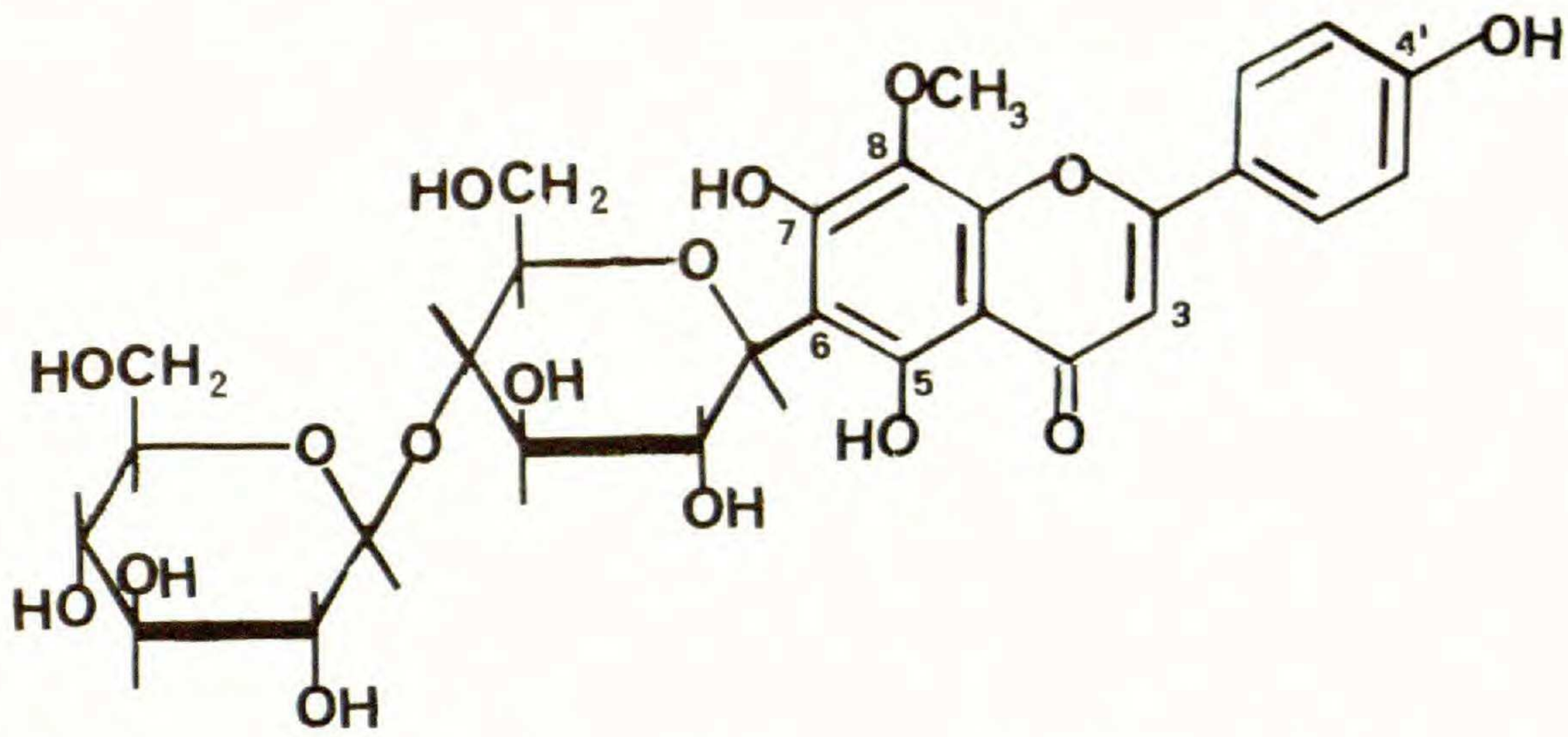

FIG. 1. Structural formula of flavonoid (I). 6-*C*-β-Cellobiosylisoscutellarein-8-methyl ether.

764) and the above UV spectral data suggested that flavonoid (I) may be a flavone *C*-diglucoside based on a flavone moiety with three hydroxyl groups and one methoxyl group. Acid hydrolysis (2N HCl/MeOH (1:1); 1 hr at 100°C) gave 2, 3, 4, 6-tetra-*O*-methyl-D-glucose and a partially methylated *C*-glycosylflavone which gave 2, 3, 6-tri-*O*-methyl-D-glucose by $FeCl_3$ oxidation. Hence a disaccharide (*O*-D-glucosyl- (1 → 4)-D-glucose) is attached to flavone moiety of I. ^{1}H NMR spectrum (DMSO-d_6) of flavonoid (I) showed signals at δ 3.12–3.91 (diglucosyl 12 protons, m), δ 3.84 (3H, s, methoxyl group), δ 4.13 (1H, d, J = 9 Hz, glucosyl anomer), δ 4.79 (1H, d, J = 9 Hz, glucosyl anomer), δ 6.81 (1H, s, H-3), δ 6.96 (2H, d, J = 8.8 Hz, H-3′ and H-5′) and δ 7.91 (2H, d, J = 8.8 Hz, H-2′ and H-6′). Since anomeric protons appeared as doublets with coupling constants J = 9 Hz, the disaccharide of flavonoid (I) is cellobiose (*O*-β-D-glucosyl-(1 → 4)-D-glucose) and this sugar is attached to the flavone moiety by a β-linkage. In addition cellobiose and methoxyl group must be on A-ring of flavone moiety since signals of H-6 and H-8 were absent in ^{1}H NMR spectrum of flavonoid (I) and B-ring protons appeared as an ortho coupled system (A_2B_2). The bathochromic shift with $AlCl_3$/HCl (51nm) of band I in UV spectrum of flavonoid (I) showed the absence of 6-oxygenation according to Markham (pp. 197–235 *in* J.B. Harborne ed., Methods in Plant Biochemistry, Academic Press, London, 1989); hence methoxyl group is at C-8 and cellobiose is attached to C-6 of flavonoid (I) which must be 6-*C*-β-cellobiosylisoscutellarein-8-methyl ether (Fig. 1), a new natural product. The following characteristics of ^{13}C NMR spectrum of flavonoid (I) supported (Table 1) this structure according to a review of Markham and Chari (pp. 19–134 *in* J.B. Harborne and T.J. Mabry eds., The Flavonoids, Advances in Research, Chapman and Hall, London, 1982). A shift (due to *C*-glucosylation) of C-6 to lower field (+9.3 ppm) in comparison with the corresponding carbon of apigenin and a shift of C-8 (due to methoxyl group) to lower field (+30.8 ppm) in comparison with the corresponding carbon of isovitexin were observed. In addition C-4″ showed

TABLE 1. ^{13}C NMR spectral data (DMSO-d_6) of flavonoid I. [a,b]Assignments with the same superscripts may be interchanged.

	Isoscutellarein-8-methyl ether		*C*-Glucosyl
C-2	163.9	C-1″	74.2[a]
C-3	101.9	C-2″	70.8
C-4	182.2	C-3″	77.1
C-5	155.6	C-4″	80.8
C-6	108.1	C-5″	79.3
C-7	153.9	C-6″	61.5
C-8	124.8		
C-9	144.1	*O*-Glucosyl	
C-10	103.1		
C-1′	121.1	C-1″′	104.4
C-2′	128.8	C-2″′	74.1[a]
C-3′	115.8	C-3″′	75.9[b]
C-4′	161.4	C-4″′	69.6
C-5′	115.8	C-5″′	76.1[b]
C-6′	128.8	C-6″′	60.8
OCH_3	59.9		

a shift to lower field (+10.1 ppm) whereas C-3″ and C-5″ showed small upfield shifts (−1.9 ppm and −2.0 pppm respectively) in comparison with the corresponding carbons of isovitexin; these shifts are due to *O*-glucosylation at C-4″. The signals of *O*-glucosyl moiety of flavonoid (I) were similar to those of the hydrolyzable D-glucose of 6-*C*-sophorosylapigenin-7-methyl ether. The following characteristic features of EI-MS of PM flavonoid (I) corroborated this structure as shown by Chopin et al. (pp. 487–490 *in* J.B. Harborne and T.J. Mabry eds., The Flavonoids, Advances in Research, Chapman an Hall, London, 1982) as well as by Bouillant et al. (Phytochemistry 17: 527–533, 1978). The presence of fragment ions at m/z 749 (M-CH_3) and m/z 733 (M-$^+OCH_3$) showed the absence of 1 → 2 interglucosidic linkage because these fragment ions are generally absent in EI-MS of 2″-*O*-glycosyl-6-*C*-glycosylflavones. The presence of a fragment ion at m/z 529 ($[S]^+$, derived from the loss of PM *O*-glucosyl moiety whitout oxygen of glucosidic bond) higher than the fragment ion at m/z 515 ($[S-14]^+$, due to elimination of C-5″ PM glucosylloxymethyl side chain) showed the absence of 1 → 6 interglucosidic link since EI-MS of PM 6″-*O*-glycosyl-6-*C*-glycosylflavone show $[S-14]^+ > [S]^+$. The base peak at m/z 383 (PM aglycone-CH = $^+OCH_3$) showed the absence of 1 → 3 integlucosidic link since EI-MS of 3″-*O*-glycosyl-6-*C*-glycosylflavones show PM aglycone-CH = ^+OH as base peak. The absence of fragment ion at m/z 545 ($[SO]^+$, derived from the loss of PM *O*-glucosyl moiety with the oxygen of glucosidic bond) as well as the absence of fragment ions at m/z 589, 575 and 559 (in which fragments of PM *O*-glucosyl moiety (-CH = $^+$OMe, -CH = ^+OH, -$^+CH_2$) are bound to $[SO]^+$) confirmed the proposed structure since these fragment ions are very weak or absent in EI-MS of PM 4″-*O*-glycosyl-6-*C*-glycosylflavones.

Flavonoid cellobiosides are rare plant constituents; in addition cellobiose

has been reported for the first time in association with fern flavonoids only recently as shown by Imperato and Telesca (above reference). According to Swain (pp. 1097–1129 *in* J.B. Harborne, T.J. Mabry and H. Mabry eds., The Flavonoids, Chapman and Hall, London, 1975) flavonoid (I) may be considered an "advanced" biochemical character from the phylogenetic point of view since a methoxyl group is present at C-8. A large number of flavonoid aglycones has been found on the outside of fronds of gymnogrammoid ferns as shown in a review of Markham (pp. 427–468 *in* J.B. Harborne ed., The Flavonoids, Advances in Research since 1980, Chapmann and Hall, 1988); some of these "external" flavonoids have an hydroxyl group (often acylated) or a methoxyl group at C-8. However hydroxyl and methoxyl groups at C-8 are near absent from "internal" vacuolar flavonoids of ferns since there is only the report of 3-*O*-glucosides of herbacetin 8-*O*-methyl ether and gossypetin 8-*O*-methyl ether from one fern species, *Humata pectinata* (Sm) Desv. (Davalliaceae) by Wu and Furukawa (Phytochemistry 22: 1061–1065, 1983). The isolation of flavonoid (I) from *Pteris vittata* represents the first occurrence in ferns of a *C*-glycosylflavone with hydroxyl or methoxyl group at C-8.

Flavonoid (II) has been identified as quercetin 3-*O*-β-glucuronide by UV spectral analysis with the customary shift reagents, total acid hydrolysis (which gave quercetin, glucuronic acid and glucuronolactone), ^{1}H NMR spectrum, ^{13}C NMR spectrum and DEPT experiments. Quercetin 3-O-glucuronide has been found previously in *Adiantum capillus-veneris* L. (Pteridaceae) by Akabori and Hasegawa (Bot. Mag., Tokyo 82: 294–299. 1969); glucuronic acid has previously been found in association with fern flavonoids only in the genus *Adiantum* as shown in the above review of Markham (1988).

Flavonoid (III) has been identified as rutin by UV spectral analysis in the presence of usual shift reagents, total acid hydrolysis (which gave quercetin, D-glucose and L-rhamnose), controlled acid hydrolysis (which gave rutinose in addition to the products of total acid hydrolysis) and co-chromatography with an authentic sample (four solvent systems); this identification was confirmed by Kuhn methylation followed by acid hydrolysis which gave 2, 3, 4-tri-*O*-methyl-L-rhamnose, 2, 3, 4, 6 tetra-*O*-methyl-D-glucose and quercetin 5, 7, 3′, 4′-tetra-*O*-methyl ether. Rutin is here reported for the first time in the genus *Pteris*; as shown in the above review of Markham (1988), rutin has previously been identified in ten species of *Adiantum* (Adiantaceae), five species of *Gymnopteris* (Sinopteridaceae), all four species of *Bommeria* (Sinopteridaceae), four species of *Hemionitis* (Sinopteridaceae), the genus *Trachypteris* (Sinopteridaceae), *Paesia anfractuosa* (Dennstaedtiaceae), *Pteridium aquilinum* (Dennstaedtiaceae) and *Loxsoma cunninghamii* (Loxsomaceae); recently rutin has been identified in *Polypodium decumanum* Wild (Polypodiaceae) by *Vasänge* et al. (Planta Medica 63: 511–517, 1997).

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